Hydroxypropyl methylcellulose-beeswax edible coatings formulated with antifungal food additives to reduce alternaria black spot and maintain postharvest quality of cold-stored cherry tomatoes

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Abstract

Edible composite coatings based on hydroxypropyl methylcellulose (HPMC), beeswax (BW), and food preservatives with antifungal properties were formulated and evaluated on cherry tomatoes during cold storage. Selected food preservatives included: sodium methyl paraben (SMP), sodium ethyl paraben (SEP) and sodium benzoate (SB). Cherry tomatoes artificially inoculated with *Alternaria alternata* were coated and stored up to 21 d at 5 °C followed by 4 d of shelf-life at 20 °C. All antifungal coatings reduced the incidence and severity of alternaria black spot on inoculated cherry tomatoes, being the SB-based coating the most effective. Analytical and sensory fruit quality was evaluated on intact and cold-stored tomatoes. In contrast to coatings containing SMP or SEP, the SB-based coating was effective to reduce weight loss and respiration rate and maintain the firmness of coated cherry tomatoes. Peel color, ethanol and acetaldehyde content of the juice, sensory flavor, off-flavors, and fruit appearance were not adversely affected by the application of the antifungal coatings. In conclusion, HPMC-BW coatings containing the food additive SB at 2% showed potential for industrial application, including the production and commercialization of organic cherry tomatoes.

Keywords: Solanum lycopersicum; edible coatings; hydroxypropyl methylcellulose; postharvest quality; food preservatives; *Alternaria alternata*

1. Introduction

Tomato fruits have a relatively short postharvest life and during fruit ripening many processes reducing fruit quality may take place, leading to important economic losses. Therefore, the development of new technologies to effectively control ripening and decay would be of great economic importance (Hoeberichts et al., 2002). *Alternaria alternata* (Fr.) Keissl., causing black spot, is among the most common fungal pathogens responsible for postharvest decay of cherry tomato fruit (Wang et al., 2008). The use of synthetic chemical fungicides as antimicrobial agents to control fungal spoilage of fresh horticultural products has been practiced for many years. However, concerns about environmental contamination and human health risks associated with fungicide residues on/in produce, as well as the proliferation of fungicide-resistant strains of the pathogens, have led to serious restrictions or even bans of many synthetic fungicides (Palou et al., 2008). At present, there is a lack of authorized postharvest treatments and/or registered fungicides available for the control of postharvest diseases of high value commercial fruits, such as tomato. Alternative methods that have been proposed for the control of postharvest diseases include biological control with antagonistic microorganisms, physical methods such as heat or radiations, and the use of low-toxicity chemicals with antimicrobial activity (Montesinos-Herrero et al., 2009; Palou et al., 2002, 2008; Valencia-Chamorro et al., 2009a). The latest include natural or synthetic compounds of known and low toxicity, usually classified as safe food-grade additives or Generally Regarded as Safe (GRAS) substances by international authorities (Larrigaudière et al., 2002; Palou et al., 2002).

In recent years, the release of antimicrobial agents incorporated into biodegradable edible films and coatings has emerged as a new, effective, and environmentally-friendly alternative mean to extend the shelf-life of many products including fresh fruits and vegetables. Edible coatings provide a semi-permeable barrier to water vapor, oxygen (O_2), and carbon dioxide (CO_2) that reduce weight loss and respiration. Additional advantages of edible coatings are the possibility to maintain the

firmness of the fruit and provide gloss to coated products (Greener-Donhowe and Fennema, 1994). Edible coatings are based on polysaccharides, proteins and lipids or a mixture of these. Other food-grade ingredients such as antimicrobial agents, antioxidants, flavors, color pigments, and vitamins can also be incorporated into the basic formulation of these coatings with the aim to improve their functional properties (Valencia-Chamorro et al., 2011a). Among the active ingredients used in antimicrobial edible coatings, compounds such as plant essential oils, food aromas, organic acids, parabens, their salts and other permitted food additives or GRAS compounds, have been preferred for fruit and vegetables (Das et al., 2013; Fagundes et al., 2013; Valencia-Chamorro et al., 2009a; Xu et al., 2007). Our research group optimized stand-alone hydroxypropyl methylcellulose (HPMC)-lipid edible composite films containing a wide variety of food additives and GRAS compounds such as mineral salts, organic acid salts and their mixtures, and sodium salts of parabens and their mixtures to provide antifungal activity against the citrus pathogens *Penicillium digitatum* and *P. italicum* (Valencia-Chamorro et al., 2008). Then, selected coatings were tested *in vivo* against green and blue molds on different citrus cultivars. The inhibitory activity of the coatings was strongly dependent on the susceptibility of each citrus cultivar to penicillium decay and the storage temperature (Valencia-Chamorro et al., 2009a, 2009b, 2010, 2011b). Similar studies also proved the antifungal activity of several mineral salts, organic acid salts, and paraben salts incorporated to HPMC-BW coatings against the pathogens *Monilinia fructicola* in artificially inoculated plums (Karaca et al., 2014) and *Botrytis cinerea* and *A. alternata* in inoculated cherry tomatoes during shelf-life at 20 ºC (Fagundes et al., 2013). In a recent work, the best coatings against *B. cinerea* were evaluated on cherry tomatoes cold-stored at 5 ºC and it was observed that the effect of the coatings on disease development and fruit quality during storage was dependent on the storage temperature, remarking the need to evaluate the coatings under commercial storage conditions (Fagundes et al., 2014). In our previous study to select appropriate antifungal coatings for the control of alternaria black spot of

cherry tomato, the best results after incubation of coated fruit at 20 ºC were obtained with HPMC-BW coatings containing 2.0% sodium benzoate (SB), sodium ethyl paraben (SEP), or sodium methyl paraben (SMP) (Fagundes et al., 2013). The objective of the present research was to determine the effect of selected HPMC-BW edible coatings formulated with antifungal food additives on the development of alternaria black spot and the physico-chemical and sensory quality of cherry tomatoes during cold storage. This information is needed for potential commercial development of suitable antifungal edible coatings.

2. Materials and methods

2.1. Materials

HPMC (Methocel E15) was purchased from Dow Chemical Co. (Midland, MI, USA). BW (grade 1) was supplied by Fomesa Fruitech, S.L. (Beniparrell, València, Spain). Oleic acid and glycerol were from Panreac Química, S.A (Barcelona, Spain). Laboratory reagent grade preservatives (99% minimum purity) were purchased from Fluka Chemie AG (Buchs, Switzerland) and Merck KGaA (Darmstadt, Germany), and included SMP ($C_8H_7NaO_3$; E-218), SEP ($C_9H_9NaO_3$; E-214), and SB ($C_7H_5O_2Na$; E-211). All these chemicals are classified as food additives (with their correspondent E-number) or GRAS compounds by the European Food Safety Authority (EFSA) and the United States Food and Drug Administration (US FDA).

2.2. Emulsions preparation

HPMC-lipid edible composite emulsions were prepared combining the hydrophilic phase (HPMC) and the hydrophobic phase (BW) suspended in water. Glycerol and oleic acid were used as plasticizer and emulsifier, respectively. All the formulations contained 30% BW (dry basis, db) and the ratios of HPMC-glycerol (3:1) (db) and BW-oleic acid (5:1) (db) were kept constant throughout the study. Tween 80 was also added to the formulations at a concentration of 1.5% (w/w) to improve wetting

of the coating and adherence to the tomato fruit. All formulations contained 2.0% (w/w) of food preservative. Emulsions were prepared as described by Valencia-Chamorro et al. (2008). Briefly, an aqueous solution of HPMC (5% w/w) was prepared by dispersing the HPMC in hot water at 90 °C and later hydration at 20 °C. The corresponding food preservative, BW, glycerol, oleic acid, and water were added to the HPMC solution and heated at 98 °C to melt the lipids. Samples were homogenized with a high-shear probe mixer (Ultra-Turrax model T25, IKA-Werke, Steufen, Germany) for 1 min at 12.000 and 23 min at 22.000 rpm. Emulsions were cooled under agitation to a temperature lower than 25 °C by placing them in a water bath and agitation was continued during 25 min to ensure complete hydration of the HPMC. The emulsions were prepared with a final solid concentration of 10% and had a viscosity in the range of 140-147 cp. Table 1 shows the viscosity and pH of the emulsions containing selected food preservatives. Emulsions were kept 1 d at 5 °C before use. These formulations were stable and no phase separation was observed.

2.3. Effect of coatings on disease development

2.3.1. Fungal inoculum

The strain TAV-6 of *A. alternata*, obtained from decayed tomato fruit in Valencia packinghouses, was isolated, identified, and maintained in the IVIA culture collection of postharvest pathogens. Prior to each experiment, the isolate was grown on potato dextrose agar (PDA; Sigma-Aldrich Chemie, Steinheim, Germany) in petri dishes at 25 °C for 7-14 d. From this culture, a high-density conidial suspension was prepared in Tween 80 (0.05%, w/v; Panreac-Química S.A., Barcelona, Spain) and sterile water. This suspension was passed through two layers of cheesecloth, measured with a haemacytometer, and diluted with sterile water to achieve an inoculum density of 1 x 10^6 spores/mL of *A. alternata.*

2.3.2. Fruit inoculation and coating application

Cherry tomatoes (*Solanum lycopersicum* L. var. *cerasiforme* cv. Josefina; syn.: *Lycopersicon esculentum* Mill.) used in the experiments were commercially grown and collected in the Valencia area (Spain) and stored up to 24 h at 5 °C until use. Fruit were free from previous postharvest treatments or coatings. Before each experiment, fruit were selected, randomized, washed with fruit biodegradable detergent at 6% (v/v) (Essasol V., Didsa, Potries, Valencia), rinsed with tap water, and allowed to air-dry at room temperature. Cherry tomatoes were superficially wounded once in the equator with a stainless steel rod with a probe tip 1 mm wide and 2 mm in length. This wound was inoculated with the pathogen by placing 10 µl of a spore suspension containing 1 x 10^6 spores/ml of *A. alternata*. After incubation at 20 °C for 24 h, inoculated fruit were coated by immersion for 30 s in the selected HPMC-BW edible composite emulsions, drained, and allowed to air-dry at 20 °C. Inoculated but uncoated fruit were used as controls. Coated fruit were placed on plastic trays on corrugated cartons and stored up to 21 d at 5 °C and 90-95% RH, followed by 4 d of shelf-life at 20 °C. In every experiment, each treatment was applied to 3 replicates of 10 fruit each. The experiments were repeated twice.

2.3.3. Determination of disease incidence and severity

The incidence of alternaria black spot was calculated as the percentage of decayed fruit. Disease severity was determined as the diameter of the lesion (mm). Incidence and severity were assessed after 7, 14 and 21 d during the storage period at 5 °C, and also after the shelf-life period of 4 d at 20 °C.

2.4. Effect of coatings on fruit quality

2.4.1. Fruit coating and storage

For the quality study, before each experiment, fruit were selected, randomized, washed with fruit biodegradable detergent, rinsed with tap water, and allowed to air-dry at room temperature. Intact and healthy fruit were divided into four groups of 120 fruit

each, which corresponded to the three coating treatments and one control (uncoated fruit). Cherry tomatoes were coated as described above, drained of excess coating, dried and stored for up to 15 d at 5 °C and 90-95 % RH. Physico-chemical and sensory fruit quality was assessed initially and after 10 and 15 d at 5 °C plus a shelf-life period of 5 d at 20 °C.

2.4.2. Assessment of fruit quality

2.4.2.1 Internal quality

The assessed internal quality attributes were soluble solids content (SSC), titratable acidity (TA), and pH of tomato juice. SSC of the juice was measured using a digital refractometer (model PR1; Atago Co. Ltd., Japan) and values were expressed as percentage. TA of tomato juice was determined by titrating 5 mL of juice sample with 0.1 M sodium hydroxide to an end point of pH 8.1 and expressed as g of citric acid per 1 L. pH of the juice was determined using a pH-meter (modelC830, Consort bvba, Turnhout, Belgium). For each treatment, 3 juice samples from 7 fruit each were prepared and three different readings were performed.

2.4.2.2. Weight loss

Lots of 30 fruit per treatment were used to measure weight loss. The same marked cherry tomato were weighed at the beginning and at the end of each storage period. The results were expressed as the percentage of initial weight lost.

2.4.2.3. Peel color

Skin color of cherry tomatoes was measured with a Minolta (Model CR-400, Minolta, Tokyo, Japan) on 20 fruit per treatment, using the CIE (Commission Internationale de l'Eclairage) color parameters lightness (L*), a*, b*, chroma (C*) and hue angle (hº). Each measurement was taken at three locations for each cherry tomato. A standard white calibration plate was employed to calibrate the colorimeter.

2.4.2.4. Fruit firmness

Firmness of 20 fruit per treatment was determined at the end of each storage period using an Instron Universal testing machine (Model 4301, Instron Corp., Canton, MA, USA). Each fruit was compressed between two flat surfaces closing together at the rate of 5 mm/min. The machine gave the deformation (mm) after application of a load of 9.8 N to the equatorial region of the fruit. Results were expressed as percentage of deformation, related to initial diameter.

2.4.2.5. Respiration rate

Respiration of coated and uncoated cherry tomatoes was measured by the closed system. Three replicates of 5 fruit each were used to determine the CO_2 production at the end of the storage period. Samples were weighed and placed in sealed containers of known volume. The accumulation of CO_2 in the headspace atmosphere was measured at 20 °C over a period of 3 h. The gas sample (1 mL) was injected into a gas chromatograph (GC) (Thermo Trace, Thermo Fisher Scientific, Inc. Waltham, MA, USA) equipped with a thermal conductivity detector (TCD) and fitted with a Poropack QS80/100 column (1.2 m × 0.32 cm i.d.). Temperatures were 35, 115, and 150 °C, respectively for the oven, injector, and thermal conductivity detector. Helium was used as carrier gas at a flow rate of 22 mL/min. The CO_2 concentration was calculated using the peak area obtained from a standard gas mixture of 15.0:2.5% O_2:CO_2. Results were expressed as mg CO_2/kg h.

2.4.2.6. Ethanol and acetaldehyde contents

Ethanol and acetaldehyde were analyzed from the head-space of tomato juice from samples using a GC (Thermo Trace, Thermo Fisher Scientific) equipped with an auto-sampler (Model HS 2000), flame ionization detector (FID), and 1.2 m x 0.32 cm (i.d.) Poropack QS 80/100 column. The injector was set at 175 °C, the column at 150

°C, the detector at 200 °C, and the carrier gas at 28 mL/min. A composite juice of three replicates of ten fruit per treatment was analyzed. Five mL of juice were transferred to 10-mL vials with crimptop caps and TFE/silicone septum seals. Samples were frozen and stored at –18 °C until analyses. A 1-mL sample of the headspace was withdrawn from vials previously equilibrated in a water bath at 20 °C for 1 h, followed by 15 min at 40 °C, to reach equilibrium in the headspace, and then injected into the GC. Ethanol and acetaldehyde was identified by comparison of retention times with standards. Results were expressed as mg of gas per 1 L of juice.

2.4.2.7. Sensory evaluation

Sensory quality of treated samples was evaluated by 10 trained judges at the end of each storage period and shelf-life (ISO8586-1:1993). Each judge was given samples from each batch and requested to evaluate flavor on a 9-point scale, where 1 = very poor and 9 = optimum, and off-flavor on a 5-point scale, where 0 = absence of off-flavor and 5 = high presence of off-flavor. Ten fruit per treatment were halved cut and separated into individual segments. Two segments from two different fruit were presented to judges in trays labeled with 3-digit random codes and served to them at room temperature. The judges had to taste the segments of each sample in order to compensate, as far as possible, for biological variation of the material. Spring water was provided for palate rinsing between samples. External aspect of the fruit (coating cracks, spots, etc.) was also evaluated by the panelists. A 3 point scale was used in which the aspect was classified as 1 = bad, 2 = acceptable, and 3 = good. Panelists were also asked to rank visually the coated fruit from highest to lowest gloss.

2.5. Statistical analysis

Statistical analysis was performed using Statgraphics 5.1. (Manugistics Inc., Rockville, MD, USA). Specific differences between means were determined by Fisher's protected least significant difference test (LSD, $P < 0.05$) applied after an analysis of

variance (ANOVA). For sensory gloss, specific differences were determined by Friedman test, which is recommended for ranking by the UNE 87023 (AENOR, 1997). For disease incidence data, the ANOVA was applied to the arcsine of the square root of the percentage of infected fruit in order to assure the homogeneity of variances. Non-transformed means are shown.

3. Results and discussion

3.1. Effect of coatings on disease development

During cold storage at 5 °C, the assayed coatings significantly reduced alternaria black spot incidence and severity compared with uncoated samples, except the SMP coating that after 14 d at 5 ºC was not effective to reduce disease incidence (Fig. 1). After 7 d at 5 ºC, control samples showed 100% disease incidence, whereas all the coatings completely inhibited mold growth. In general, the reduction of disease severity by coating application was considerably higher than the reduction of disease incidence. At the end of the 21-d cold storage period, the SEP and SB coatings were the most effective to reduce the severity of black spot (reduction of around 65%). These coatings also reduced disease incidence effectively, being those containing SB the most effective (reduction of around 70% and 30% after 14 and 21 d of storage, respectively). When tomatoes were transferred to 20 °C to simulate shelf-life, the coatings did not prevent the onset of disease, and black spot incidence basically reached 100%. This result might have been influenced by the high concentration of fungal inoculum that was used in these trials (10^6 spores/mL). This high inoculum density of *A. alternata* was used to obtain high percentages of decay on control fruit and to conservatively select only those coatings with the highest potential for effective commercial usage. From results of disease incidence and severity, it can be confirmed that the activity of edible coatings was fungistatic rather than fungicidal, because mold growth was delayed but not completely inhibited and both incidence and severity increased with storage time. In general, comparable differences in performance

depending on the amount of fungal inoculum, fruit cultivars or fruit characteristics have been observed with most of the alternative antifungal treatments, which mode of action is rather fungistatic than fungicidal (Palou et al., 2008).

Among the three food additives tested as antifungal coating ingredients, SB was the most effective against *A. alternata*, followed by SEP, whereas SMP lost effectiveness after 14 d at 5 ºC (Fig. 1). SB is among the most widely used antimicrobial food additives. Its antimicrobial activity is pH-dependent, being the undissociated form the most effective. Therefore, the use of this additive is recommended in acidic products such as citrus fruits, and good disease control performance has been reported with SB applied both as aqueous solution (Palou et al., 2002) and as a fruit coating ingredient (Valencia-Chamorro et al., 2010). Parabens and their sodium salts are GRAS compounds with increasing interest as alternative low-toxicity means to control postharvest decay in fresh horticultural products. For instance, satisfactory control of citrus postharvest diseases was observed when SMP and SEP were included in the formulation of antifungal edible coatings (Valencia-Chamorro et al., 2009a) and also when they were applied as postharvest dip treatments (Moscoso-Ramírez et al., 2013a, 2013b). Parabens are in the undissociated form at pH values of most foods (p*K*a = 8.5) and are effective over a wide pH range of 4–8 (Thompson, 1994). Paraben salts like SMP and SEP are more soluble in water than their correspondent parabens and they might interfere on both the germinative and vegetative phases of microbial development, although it has been reported that fungal spore germination is much more susceptible than vegetative growth (Watanabe and Takesue, 1976). It has been suggested that the general mode of action of these salts is through an uncoupling of oxidative phosphorylation, inhibition of NAD+ and FAD-linked mitochondrial respiration, or the reduction of mitochondrial membrane potential (Soni et al., 2001).

3.2. Effect of coatings on fruit quality

3.2.1. Fruit internal quality

The antimicrobial HPMC-BW coatings did not significantly affect SSC, TA and pH of cherry tomatoes (Table 2). During storage and shelf-life, SSC increased and TA decreased with respect to the values at harvest, as a consecuence of an increase in the content of soluble sugars and a decrease in the amount of organic acids. In general, the effect of coating application on fruit internal quality parameters has been proven to be dependent on coating type, fruit cultivar and storage conditions. While some authors found no differences in these parameters after coating application on different citrus cultivars (Baldwin et al., 1995; Obenland et al., 2008), others observed lower SSC and TA reductions when compared to uncoated controls, which was always related to decreases in weight loss and respiration rate (Togrul and Arslan, 2004). In some works with tomatoes, the application of edible coatings resulted in lower SSC (Ali et al., 2010; Yaman and Bayoindirli, 2002) and TA (Das et al., 2013) than in uncoated samples, which was attributed to the fact that the coatings provided a semi-permeable barrier to gases around the fruit, modifying the internal atmosphere by reducing O_2 and/or elevating CO_2 and suppressing ethylene production.

3.2.2. Weight loss

Fig. 2 shows the weight loss on coated and uncoated samples stored for 10 and 15 d at 5 °C, followed by 5 d of shelf-life at 20 °C. Tomatoes are naturally covered by a continuous wax layer that provides high resistance to water movement across the cuticle. Coatings containing hydrophobic compounds, deposited as an additional layer over the natural waxes, should improve the moisture resistance of the fruit (Fagundes et al., 2014). In our work, the barrier properties of the coatings were greatly influenced by the different food additives incorporated to the HPMC-BW matrix. Whereas SB slightly but significantly reduced tomato weight loss ($P \leq 0.05$), SEP and SMP increased it compared to uncoated samples. This might indicate a partial removal of the natural waxes present on the peel or a negative interaction of these particular salts

with the waxes. According to the literature, edible coating application has significant or not significant effects on weight loss of fruits depending on intrinsic characteristics of both the coating and the fruit. Thus, for example, the addition of lipids to polysaccharides did not reduce weight loss of coated commodities, such as cherries or cucumbers (Baldwin et al., 1997), apples (Bai et al., 2002), or plums (Navarro-Tarazaga et al., 2008). In addition, several works confirmed that the addition of food preservatives to HPMC-based coatings greatly affects the moisture barrier properties of stand-alone films or coatings when applied to different fruits such as citrus, table grapes or cherry tomatoes (Fagundes et al., 2014; Pastor et al., 2011; Valencia-Chamorro et al., 2008, 2009a). Furthermore, weight loss after the application of some HPMC-based coatings clearly depended on the commodity and cultivar. For instance, HPMC-lipid coatings containing organic acids salts and their mixtures significantly reduced weight loss of coated 'Clemenules' and 'Ortanique' mandarins during long-term storage, but did not reduced that of 'Valencia' oranges, and some coatings even induced a significant increase in weight loss of this cultivar (Valencia-Chamorro et al., 2009b, 2010, 2011b). In previous research with cherry tomatoes, the addition of sodium propionate and potassium carbonate to similar HPMC-based coatings also increased weight loss compared to uncoated samples (Fagundes et al., 2014). This was correlated with the intrinsic moisture barrier of the coating, but also with the mechanical properties that might affect coating integrity during prolonged storage of the fruit. Compared to the properties of stand-alone films, coating performance is affected by coating distribution over the fruit surface, especially whether it forms a continuous layer or penetrates into pores. Moreover, fruit skin morphology (presence of hairs, thickness and type of cuticle, number of stomata, lenticels, and even cracks in the lenticels) and coating physical properties such as surface tension and viscosity strongly influence mass transfer of the coated fruit (Hagenmaier and Baker, 1993). For these reasons, beyond *in vitro* determination of film properties, the evaluation of coating

performance in *in vivo* trials with target commodities is mandatory to assess the actual potential for industrial application of novel fruit coatings.

3.2.3. Peel color

The color change that accompanies maturation in many fruits is one of the most important quality criteria used by growers and consumers to judge the harvest time and the commercial quality. Thus, in tomatoes, the red color is the most visible and important quality attribute for marketing. This color is the result of a combination of carotenoid pigments, being lycopene (red) the most abundant, followed by carotenes (yellow to orange) and xanthophylls (yellow) (López et al., 2001). Table 2 shows the CIE color parameters of coated and uncoated cherry tomatoes after 15 d of storage at 5 °C plus 5 d at 20 °C. In uncoated samples, there was a decrease in L^*, b^* and h° values with storage time. At the end of storage, the coatings helped to maintain b^* and h° values, whereas coated tomatoes presented lower L^* than uncoated ones and no differences were observed in a^* values among treatments. The differences in b^* values between coated and uncoated tomatoes translated into lower h° (which indicates more reddish tonalities) and a slight decrease in C^* (purity or saturation of a single color) in uncoated than in coated samples. Several works reported a delay in color changes in tomatoes during storage at 20 ºC by coating application related to its capacity to create a modified atmosphere in the fruit (Ali et al., 2010; Zapata et al., 2008; Zhuang and Huang, 2003). However, we reported in previous research (Fagundes et al., 2014) that although the use of HPMC-BW coatings with different food preservatives reduced respiration rate in cherry tomatoes, their effect was insufficient to produce a significant change in the peel color of coated cherry tomatoes during cold storage. Furthermore, the initial full-developed red color of the cherry tomatoes used in this study (Table 2) could also explain the small changes observed in color during cold storage and the shelf-life storage period of 5 d at 20 ºC.

3.2.4. Fruit firmness

Firmness values, expressed as percentage of deformation, of coated and uncoated samples are shown in Fig 3. No significant differences were observed between cherry tomatoes coated with the SB-based coating and the uncoated control, whereas the samples treated with the SMP and SEP-based coatings presented higher deformation values and consequently lower firmness than the control. The effect of coatings on the maintenance of fruit firmness is usually related to their control of weight loss and/or the modification of the internal atmosphere of the fruit (Baldwin et al., 1997; Seymour et al., 1993; Yaman and Bayoindirli, 2002). Thus, firmness retention in coated tomato has been repeatedly related to a reduction in enzymatic activities caused by a modification of the internal atmosphere of the fruit (Ahmed et al., 2013; Ali et al., 2010; Park et al., 1994; Zapata et al., 2008; Zhuang and Huang, 2003). In this work, cherry tomatoes coated with SEP-based HPMC-BW coatings had higher respiration rate than control and the rest of coated samples during storage (Fig. 4), which could be related with lower fruit firmness. However, this relationship was not observed for SMP-coated samples. In any case, the samples with the lowest firmness (fruit treated with coatings containing SMP and SEP) suffered the highest weight loss (Figs. 2, 3).

3.2.5. Respiration rate

The effect of the coatings on respiration rate of cherry tomatoes during cold storage plus 5 d at 20 °C is shown in Fig. 4. In general, there was an increase in the respiration rate of cherry tomatoes during storage, which indicates an increase in the fruit metabolic activity. Ideally, the effect of coatings on respiration of horticultural products is related to their ability to create a barrier to O_2 diffusion through the coating, which translates in lower respiration rates in coated fruits (Fagundes et al., 2014). However, the capacity to create this O_2 barrier greatly depends on the amount and nature of minor ingredients in the coating formulation. Additional ingredients, such as antioxidant or antimicrobial food additives, can modify their effectiveness (Valencia-

Chamorro et al., 2008). In this work, only the tomatoes coated with the SB-based coatings showed lower respiration rates than uncoated samples, whereas SEP-coated tomatoes had the highest respiration rates. Valencia-Chamorro et al. (2008) reported a 10-fold increase in O_2 permeability of HPMC-lipid edible films amended with SEP compared to SB-based films, which could explain the differences in respiration rate induced by these formulations when applied to cherry tomatoes.

3.2.6. Ethanol and acetaldehyde content

The application of HPMC-BW coatings increased ethanol content in the juice of coated cherry tomatoes ($P < 0.05$; Fig. 5). Overall, the concentrations of acetaldehyde and ethanol in the juice of coated cherry tomatoes after both storage periods were in the range of 0.76-1.30 mg/L and 4.2-13.09 mg/L, respectively, while they were in the range of 0.43-0.55 mg/L and 0.32-0.66 mg/L, respectively, in uncoated samples. These values were higher than those reported in previous work by Fagundes et al. (2014), when similar HPMC-based coatings amended with different antimicrobial agents were applied to cherry tomatoes, showing the importance of the role that minor ingredients may play in the final performance of edible coatings. In general, the effect of the application of coatings amended with SMP or SEP on quality parameters like weight loss, respiration rate, and firmness indicates that these antifungal additives affected negatively the metabolism of cherry tomatoes, accelerating the metabolic activity and increasing the production of volatile compounds. However, as it will be discussed in the next subsection, the increment of these volatiles in the juice of cherry tomatoes was not high enough to induce noticeable off-flavors in coated fruit.

3.2.7. Sensory evaluation

The HPMC-BW based coatings containing food preservatives did not modify the flavor of cherry tomatoes compared to uncoated samples. The panelists considered the flavor as acceptable irrespective of the treatments and the storage time. At the end of

the storage period, after 15 d of storage at 5 °C plus 5 d at 20 °C of shelf-life, flavor scores were around 5.6-6.4 (considered as acceptable) and no differences were detected among coated and uncoated samples (data not shown). In this study, the panelists detected a slight off-flavor after storage (0.6-0.9) but no differences between coated and uncoated samples were observed, which indicates that it was not due to coating application but to storage time. It can be deducted from these results that the ethanol and acetaldehyde levels reached after cold storage at 5 ºC plus 5 d at 20 ºC were below the threshold of off-flavors detection for cherry tomatoes. Ali et al. (2010) reported that flavor and overall acceptability of tomatoes coated with gum arabic depended on the solid content of the formulation. Thus, only tomatoes coated with 10% gum showed the highest scores in all parameters after 20 d of storage, while tomatoes coated with 15 or 20% gum presented off-flavors and were not acceptable by the panel of experts.

In this work, the addition of selected food preservatives to HPMC-BW emulsion resulted in stable emulsions. After storage, all coated samples were evaluated as acceptable, although fruit coated with the SEP-based coating were rated as those with the best external appearance after 15 d at 5 ºC plus 5 d of shelf-life at 20 °C (data not shown). After both storage periods, none of the tested coatings provided higher gloss than the uncoated control. Similar results were reported by Fagundes et al. (2014) working with HPMC-BW coatings amended with other antifungal compounds like potassium carbonate and sodium propionate that had been selected for their antifungal activity against *B. cinerea*, the cause of tomato postharvest gray mold. This behavior was related to the macroemulsion character of coating formulations (Hagenmaier and Baker, 1994).

4. Conclusion

During cold storage at 5 °C, all the coatings reduced black spot incidence and severity compared with uncoated samples, and the SB-based coating was the most

effective at inhibiting the development of *A. alternata*. When tomatoes were transferred to 20 °C to simulate shelf-life, the coatings did not prevent the onset of disease in these artificially inoculated fruit, but significantly reduced disease severity. The SB-based coating was also effective to reduce weight loss and respiration rate of cherry tomatoes, whereas the sodium salts of paraben tested negatively affected these quality parameters. None of the antifungal coatings affected negatively the color, sensory flavor, off-flavor, and fruit appearance. Overall, HPMC-BW edible composite coatings containing SB as antifungal agent could be a promising industrial treatment to control black spot and maintain the postharvest quality of tomatoes destined to both domestic or export markets. Since SB is a common food additive, the use of these edible coatings could be also of interest for organic producers. Further research should focus on the improvement of the physical characteristics of these HPMC-BW edible composite coatings in order to increase water loss control and enhance gloss and visual quality of coated fruit. Moreover, future work should also consider testing the selected SB coating on other cultivars and types of tomato fruits in order to widen its potential commercial usage.

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Table 1. Characteristics of hydroxypropyl methylcellulose-beeswax edible composite coatings containing antifungal food preservatives

Food preservative	E-number	Molecular formula	Solid concentration (%)	Viscosity (cp)	pH
Sodium methyl paraben	E-218	$C_8H_7NaO_3$	10.0	140.4	9.60
Sodium ethyl paraben	E-214	$C_9H_9NaO_3$	10.0	147.0	9.70
Sodium benzoate	E-211	$C_7H_5O_2Na$	10.0	142.3	6.39

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664**Table 2.** Soluble solid content (SSC), titratable acidity (TA), pH and peel color (CIE parameters)

665of cherry tomatoes coated with hydroxypropyl methylcellulose-beeswax edible composite

666coatings containing antifungal food preservatives and stored at 5 °C for 15 d followed by 5 d of

667shelf-life at 20 °C.

	Quality attributes							
Food preservative	pH	SSC (%)	TA (g citric acid/L)	*L**	*a**	*b**	*C**	*hº*
Control[a]	4.47 a	7.27 a	4.05 ab	35.60 a	15.23 a	18.04 a	23.63 a	49.86 a
Sodium methyl paraben	4.47 a	7.15 a	4.04 ab	33.96 b	14.89 a	19.59 b	24.64 ab	52.83 b
Sodium ethyl paraben	4.45 a	6.77 a	4.15 b	34.48 b	14.63 a	19.86 b	24.73 b	53.75 b
Sodium benzoate	4.54 b	7.07 a	3.84 a	34.38 b	15.00 a	19.47 b	24.63 ab	52.54 b

668Values at harvest: TA = 4.63 g citric acid/L; SSC = 6.48%; pH = 4.32; *L** = 36.85; *a** = 14.47;
669*b** = 19.32; *C** = 22.71; *hº* = 54.10

670[a] Control = uncoated.

671Means in columns with different letters are significantly different according to Fisher's protected
672LSD test ($P < 0.05$) applied after an ANOVA.

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Figure captions

Fig. 1. Disease incidence and severity (±SD) of black spot on cherry tomatoes artificially inoculated with *Alternaria alternata*, uncoated (Control) or coated 24 h later with hydroxypropyl methylcellulose-beeswax edible composite coatings containing: sodium methyl paraben (SMP), sodium ethyl paraben (SEP) or sodium benzoate (SB), stored at 5 °C for up to 21 d followed by 4 d of shelf-life at 20 °C. For each storage period, columns with different letters are significantly different by Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA. For disease incidence, the ANOVA was applied to arcsine-transformed values. Non-transformed means are shown.

Fig. 2. Weight loss of cherry tomatoes uncoated (Control) or coated with hydroxypropyl methylcellulose-beeswax edible composite coatings containing sodium methyl paraben (SMP), sodium ethyl paraben (SEP) or sodium benzoate (SB), stored at 5 °C for 10 or 15 d followed by 5 d of shelf-life at 20 °C. For each storage period, columns with different letters are different by Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA.

Fig. 3. Firmness of cherry tomatoes uncoated (Control) or coated with hydroxypropyl methylcellulose-beeswax edible composite coatings containing: sodium methyl paraben (SMP), sodium ethyl paraben (SEP) or sodium benzoate (SB), stored at 5 °C for 10 or 15 d followed by 5 d of shelf-life at 20 °C. For each storage period, columns with different letters are different by Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA.

Fig. 4. Respiration rate of cherry tomatoes uncoated (Control) or coated with hydroxypropyl methylcellulose-beeswax edible composite coatings containing, sodium methyl paraben (SMP), sodium ethyl paraben (SEP) or sodium benzoate (SB), stored at 5 °C for 10 or 15 d followed by 5 d of shelf-life at 20 °C. For each storage period,

columns with different letters are different by Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA.

Fig. 5. Ethanol and acetaldehyde content in the juice of cherry tomatoes uncoated (Control) or coated with hydroxypropyl methylcellulose-beeswax edible composite coatings containing sodium methyl paraben (SMP), sodium ethyl paraben (SEP) or sodium benzoate (SB), stored at 5 °C for 10 or 15 d followed by 5 d of shelf-life at 20 °C. For each storage period, columns with different letters are different by Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA.

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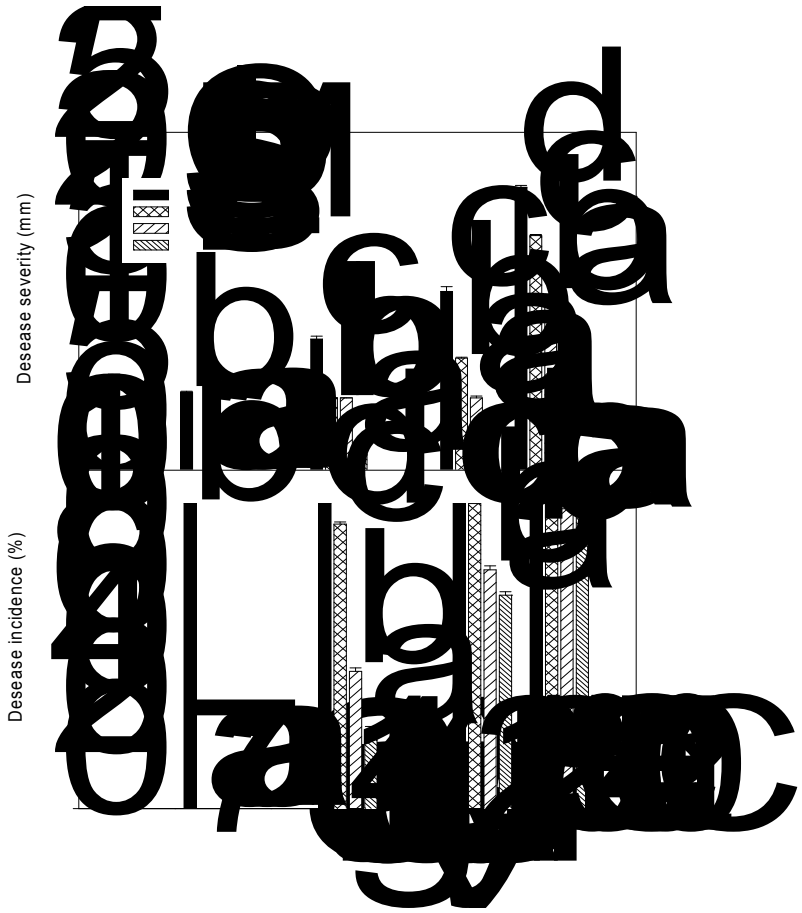

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722 **Figure 1**.
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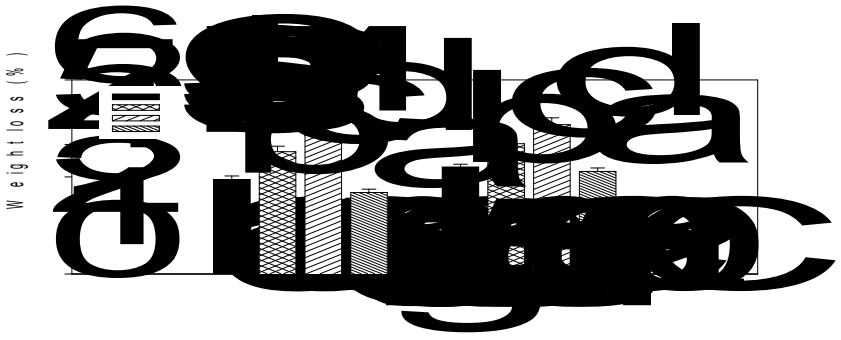

Figure 2.

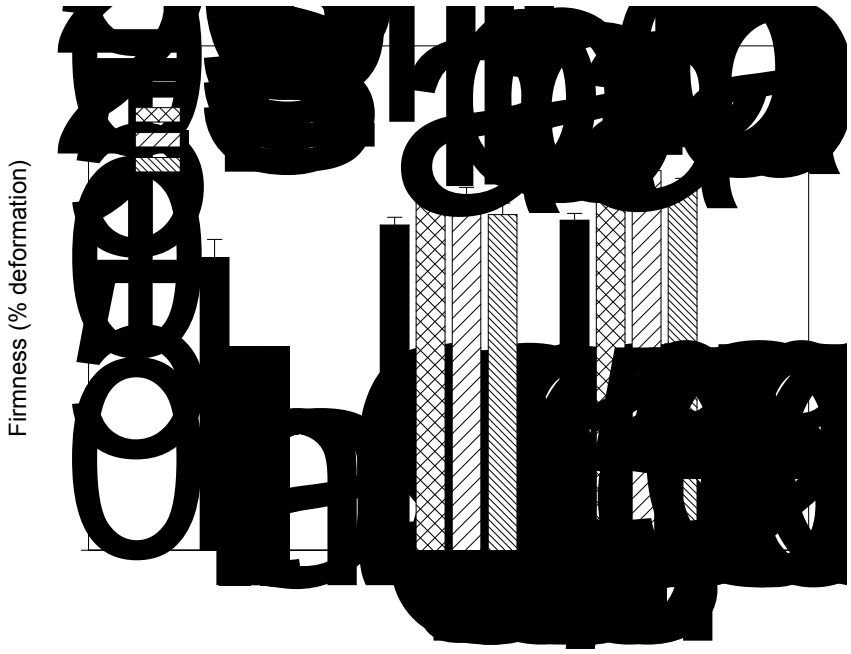

Figure 3.

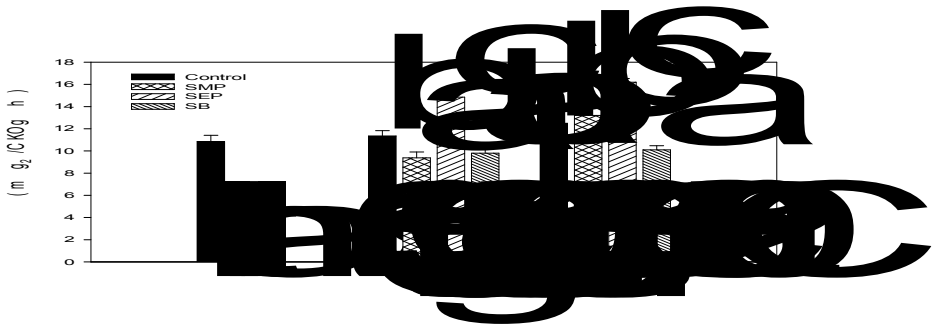

Figure 4.

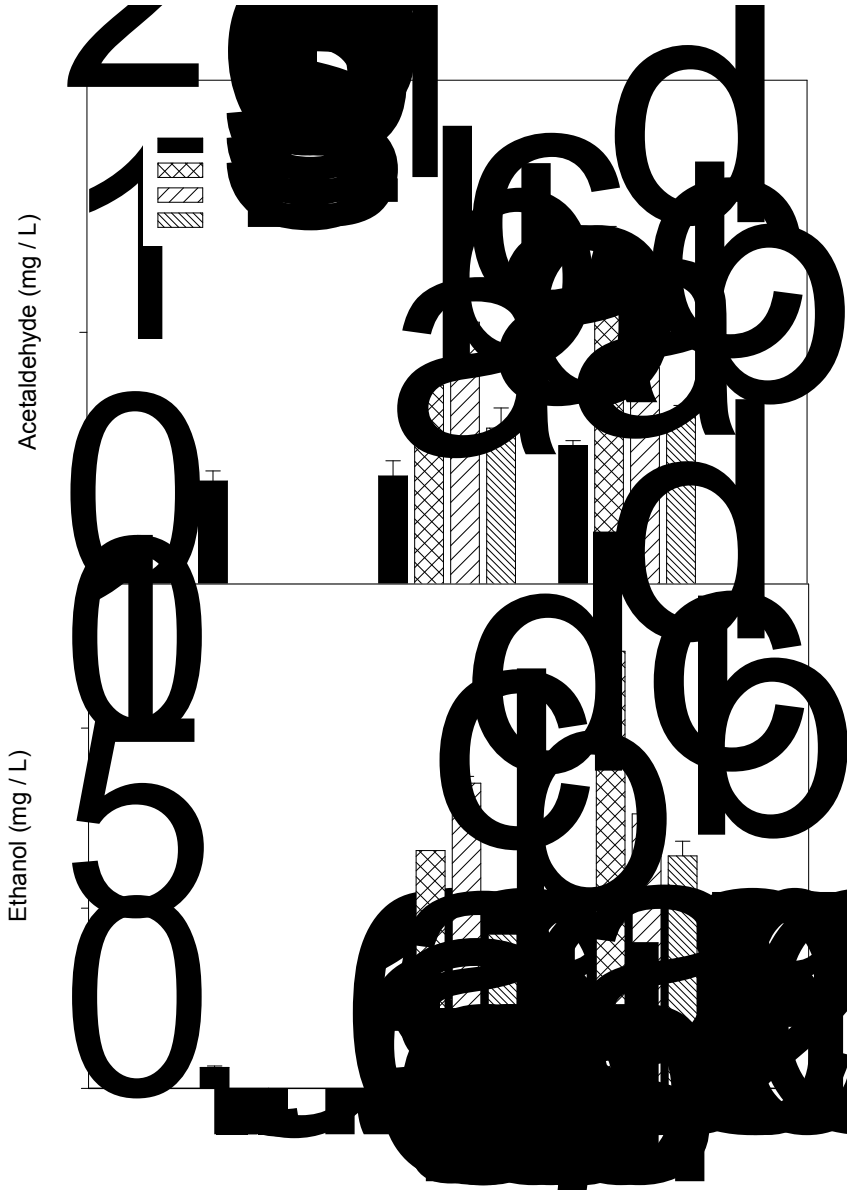

Figure 5.